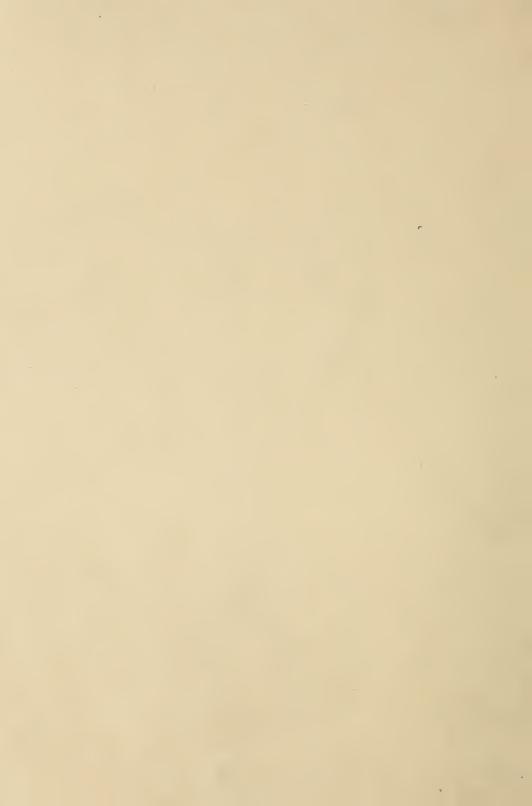
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PORTO RICO AGRICULTURAL EXPERIMENT STATION

D. W. MAY, SPECIAL AGENT IN CHARGE

MAYAGUEZ JULY 1909.

CIRCULAR No. 11.

SOIL DISINFECTION IN AGRICULTURE

BY

OSCAR LOEW.

OFFICE OF EXPERIMENT STATIONS.

11. 5. Department of Agriculture.

MAYAGUEZ. P. R.

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1909.

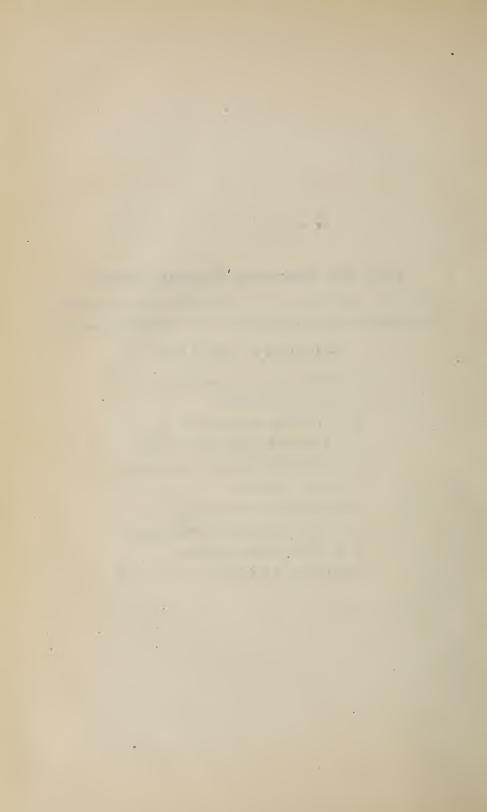
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Porto Rico Agricultural Experiment Station.

(Under the supervision of A. C. TRUE, Director of the Office of Experiment Stations, United States Department of Agriculture.)

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SOIL DISINFECTION IN AGRICULTURE

It has long been recognized that plants carry on a respiration process like animals. The plant organs above the ground are very favorably situated in this regard, fresh air circulating continuously around them, providing oxygen, which is absorbed through the stomata. (1) But the subterranean organs, as roots and certain kinds of stems, are often less favorably situated, inasmuch as only very loose soils will admit of a ready renewal of air, while stiff tenacious soils will only slowly exchange fresh air against the accumulated carbonic acid produced by the respiration of the roots. Thus, with a depression of the respiration of the roots their useful functions may be impaired. Plants with hollow stems such as rice, are in this respect, favorably constructed in that they can provide the roots with fresh air through the stem. Now it must also be taken into consideration that by manuring with organic compounds as tankage, blood meal, or animal dung, also by the decay of dead vegetation, very favorable nutrients are furnished for the bacterial life in the soil, which may thereby increase enormously. While uncultivated sandy soils contain, according to Houston, on an average 100,000 bacteria per gram and forest soils 3-500,000, manured garden soils often show a content of 1,500,000 and sewage contaminated soils 115,000,000 per gram. The abundance of microbes decreases with the depth and even at one meter comparatively few may be present. Nevertheless there may be a rapid consumption of oxygen from the air contained in the cracks and pores of the soil by the respiration process of the microbes; also replacement of that oxygen by carbonic acid produced through respiration and fermentative activity of these microbes.

Stoklasa (2) has determined that the bacteria in common field soils produce daily per hectar to a depth of 40 cm 75 kilos of carbonic acid, while an equal area of wheat, by the respiration of its roots, produces only 60 kilos. (3). Nobody can deny that this carbonic acid before it escapes into the atmosphere can act tavorably on the disintegration of the rocky particles of the soil dissolving carbonates and phosphates of lime and magnesia

⁽¹⁾ In daylight, of course, the oxygen liberated by the chloroplasts can serve directly for respiration.

⁽²⁾ Centralbl. Bakt., 1905, pp. 735.
(3) It has long been known that the air in soils is richer in carbonic acid than the air above it. The mass and activity of microbes in a given soil might be approximately estimated by the determination of carbonic acid produced under certain conditions.

for the needs of the plant; but on the other hand the healthy

respiration of the root may be interfered with.

From this point of view the disinfection of a soil overcharged with microbes must show a very beneficial effect on the health of the roots and consequently upon the well being of the entire plant, since after the microbes have been killed, they no longer use up the oxygen and manure. The continuous struggle for air and food between the roots on the one side and microbes on the other is

thus interrupted in favor of the plant.

It was long ago observed that by burning the soil its productivity could be much increased, especially with clay or loam. This was formerly ascribed wholly to the improvement of the mechanical condition. However, the destruction of bacterial life thereby counts no doubt for something. The burning process brought doubtless several advantages at the same time, although occasional disadvantages as the production of an alkaline reaction must not be overlooked but properly attended to. Mixing or burnt and unburnt soil gives sometimes the best results. When humous soils are heated moderately, volatile acids may be formed from humus, and in such cases the full benefit of sterilizing be obtained only after neutralization of the acidity by moderate liming.

For sterilization of small lots of soils steam has recently been

applied also, but this treatment is not economical. (1)

The most recent and notable progress in this direction was made by the application of chemicals with disinfecting properties and this was of special benefit in so far as the nitrogen of the soil was not lost thereby as in burning the soil.

This movement was inaugurated by the treatment of soils with carbon bisulphid which was originally applied in vineyards merely with the intention to kill parasites, as Phylloxera.

a very favorable effect was observed even in cases where no parasites at all had been present.

This led in recent years to trials with other disinfectants. Thus, formalin in a strength of 2 pints to 50 gallons of water was applied to the soil at the rate of one gallon per square foot whereby microbes and animal parasites were equally well destro-Various products of tar distillation were also tried with success, as carbolic acid, creolin, and carbolineum, the latter two containing antiseptics, as cresols dissolved in hydrocarbons. Chloroform, ether, and benzene, have also been applied, but their high price forbids their practical application.

The extended pratical use of carbon bisulphid in recent years has produced such striking results that a search was induced for other reasons for its beneficial action beside that of the mere amprovement in conditions for root respiration. The effect was equal to that of an increased natrition with nitrogen. Some source of

⁽¹⁾ The cost has been estimated by Stone at \$2 for

⁽²⁾ Nematodes were thus easily destroyed but not their eggs. The cost of this treatment has been estimated at \$2 per bed 50 ft. long and 6 ft wide

nitrogen seemed to have been suddenly opened by the disinfection. Hiltner and Stoermer, who carried on a series of careful bacteriological investigations on this question, arrived at the following conclusions which were confirmed afterwards by Heinze; viz, that there exists in manured soil an equilibrium between the different kinds of soil bacteria, which is disturbed by the disinfectant, most bacteria being killed and only a few resistant species surviving. The former cause various fermentations (as for example the fermentation of Pectin) which injure germinating seeds; while the latter have mostly a beneficial action and undergo a most rapid multiplication when the disinfectant has disappeared by gradual volatilization. To the noxious kinds destroyed belong the denitrifying microbes which decompose nitrates with formation of free nitrogen. Further, nitrifying microbes are decreased considerably at first, but they may later increase very rapidly as the extent of nitrification sometimes shows. (1) To the kinds that survive belong the nitrogen fixing bacteria, Azotobacter, the nodule forming bacteria infecting the roots of leguminous plants, and certain microbes yielding ammonia from other compounds. Heinze observed an increase of nitrogen in these soils.

The bacterial flora of the untreated soil serving for the investigations of Hiltner and Stormer consisted of about 20 per cent of Streptothrix, 75 per cent of species not liquifying gelatine and 5 per cent of species that lifiquify gelatine. By treatment of this soil with the usual doses of carbon bisulphid nearly three fourths of all the microbes were killed, among which above all were most kinds of

Streptothrix.

Heinze (2) observed a very injurious action of carbon bisulphid on the development of Proteus vulgaris and Bacterium fluorescens, two kinds of microbes concerned in the putrefaction of proteins; and also on those microbes which produce acids by fermentative action, as certain species of Clostridia and Plectridia. Mold fungi as well as such lower algae ocurring in soils, as Chlorophyceae and Cyanophyceae (Nostocaceae), are more resistant to carbon bisulphid than various kinds of bacteria.

A theory different from that of Hiltner was suggested by Koch who ascribed the favorable effect of disinfectants to a kind of stimulation of growth by traces of the disinfectant remaining in the soil. It cannot be denied that, according to many observations injurious compounds can act es stimulants when appiled in relatively very small doses. Indeed our own experiments have also shown that traces of bisulphid can exert stimulating action, but this is quite insufficient to explain the powerful effects of larger doses on soils overcharged with microbes.

Moritz and Scharpe (3) expressed the opinion that carbon bi-

⁽¹⁾ The nitrification process is doubtless useful, but not absolutely necessary for plants; it may also be absent especially, in acid soils. Besides, nitrification often leads to loss of nitrogen by leeching.

⁽²⁾ Centralblatt für Bakteriologie, II Abt. vol 16, pp. 329 (1906,) (3) Arbeiten d. Kais. Gesundheitsamts vol. III, Berlin (1906.)

sulphid may gradually undergo an oxidation in the soil, furnishing thereby carbonic and sulphuric acids, the latter having a dissolving action on rock particles thus unlocking potassa and phosphoric acid. But since carbon bisulphid is not subject to spontaneous oxidation it would have to be assumed that certain surviving bacteria cause that oxidation which would be highly improbable. It is true, Heinze has observed a slight increase of sulphuric acid in soils treated with carbon bisulphid, but this might have been due to the oxidation of sulphur derived from the proteins of the killed bacteria.

Heinze as well as Pickering (1) have observed an increase of soluble matter, partly organic, partly inorganic as an effect of the soil treatment with disinfectants, but they did not furnish an explanation of this interesting fact. Pickering mentions merely that the treatment of soils with antiseptics induces a "change equivalent to that obtained by heating the soil to 75 degrees C and this may be sufficient to account for the increased growth observed in plants grown in them." He further states that organic matter became

gradually again insoluble.

The most probable explanation for that production of soluble organic matter is furnished by the fact well known in plant physiology to which the writer has called attention, namely: "Whenever a cell is killed, the cytoplasma becomes permeable for all compounds that were present in the dissolved state in the cells and hence pass to the outside." Therefore, the microbes of the soil will, in the moment of death, lose all their dissolved matter comprising chiefly nitrogenous compounds and potassium phosphate. In order to obtain some quantitative data along this line an experiment was carried out with yeast cells (2), which chemically much resemble microbes although belonging to a different group of fungi. Moist yeast 63,27 grams, corresponding to 14,42 grams dry matter was mixed with 120 cc water and 20 cc carbon bisulphid and with frequent shaking let stand for 17 hours. By this treatment all yeast cells were killed. The filtrate consisted of organic and inorganic matter, the latter containing 0,167 grams P2O5 and 0,165 grams K2O. On further examination it was found that the killed yeast cells had lost 20,50 per cent of their dry matter (partly peptones) and 69 per cent of their total mineral content (chiefly dipotassium phosphate). The dry matter lost contained one-fifth of the total nitrogen of the cells. Hence the appearance of soluble nitrogenous and mineral matter after soils, enormously rich in microbes, have been desinfected or heated to 75° C. is not surprising. Not only microbes, but also mold fungi, various algae and insects killed by the disinfectant will give out soluble matter to the soil, if there is no cuticula to prevent it.

But how can dead microbes be utilized? There is doubtles still a considerable amount of nitrogen in the form of proteins stored

⁽¹⁾ Journ. Agr. Sc., 3. No. 1, 1908. (2) O. Loew and K. Aso, On Changes of Availability of Nitrogen in Soils, Bul. College of Agriculture, vol VIII, No. 3 and 5, Tokyo 1907.

up in these dead microbes after their soluble matter has been lost. This question is probably best explained by the fact that certain bacteria can produce enzyms capable of dissolving microbes, (1) which process will be more easily performed when the latter have been killed.

Π

PRACTICAL EXPERIENCE.

Oberlin was the first who observed the favorable effect of carbon bisulphid on plant growth while he was engaged in its application against the Phylloxera of the grape. He then recommended carbon bisulphid also for cases in which the soil ceased to produce in spite of rich manuring and absence of parasites. Such soil was termed "tired" or "sick" and here an application of bisulphid effected a restoration to a healthy condition, rendering probable the supposition that, under certain conditions, the bacterial mass of the soil had increased to an unhealthful amount. The application of bisulphid was found favorable not only in cases of a decided "soil sickness" but also in various non-sick fields that had been for years organically manured and that contained much organic matter. Moritz and Scherpe, who treated a field with carbon bisulphid, on which potatoes were planted afterwards, obtained 190 parts of tubers in the treated soil and 100 parts in the untreated soil. Koch carried on experiments with buckwheat and observed a harvest of 294 grams on a plot treated with bisulphid while there were only 162 grams. on the check plot.

Behrens cultivated onions in a "tired" soil; the treatment with bisulphid having taken place the previous autumn. The plots meas-

ured to square meters each. The result was as follows:

Bisulphid cc applied per sq. meter.	Yield in kilos.	
0	14	
400 800	22	
800 1200	22 26	

Mach applied bisulphid at a rate of 200 cc per sq meter and observed considerable increase in the yield of oats, corn, potatoes and beets (L'engrais, 1896).

Girard applied bisulphid in doses of 33 kilos per hectar and obtained a yield amounting to more than double that of the control plot.

⁽¹⁾ Cf. O. Loew and R. Emmerich, Zeitschrift f. Hygiene 1899 and O. Loew and K. Aso, Bul. College of Agriculture Vol. VIII, Tokyo 1908.

A doubling of the yield by this treatment was also observed by Hiltner and Störmer with buckwheat grown on "pea tired" soil, while a further growth of peas on the same soil showed noticeably

less favorable results.

Nobbe and Richter observed a considerable increase of peas and oats after treatment of the soil with bisulphid, ether, chloroform, benzene or hydrogen peroxide. Kozai also applied formalin successfully. It is self-evident that the bisulphid treatment would not continue to produce an increase for a series of years. Manuring must be renewed after the nutrients that were stored in the bodies of dead microbes are consumed, but bisulphid may be again applied after a series of years when the microtes have once more accumulated in the soil. Wollny showed that two and three years after the bisulphid treatment the yields of field crops were smaller on unmanured soils than on those which had not received an application of bisulphid. His field experiment plats were 4 sq. meters in area, each plat being provided with 16 holes, equally distant from each other and from 30 to 50 cm in depth. He applied 25 cc of carbon bisulphid in each hole While bisulphid applied some months before planting was favorable, that applied directly to the growing plants proved injurious, they being either killed or retarded in growth. It is different, however, when bisulphid is applied to large trees, 100 to 400 c.c. of which may be safely used according to the size of the tree and the distance from the trunk at which the bisulphid is injected.

A soil may be "sick" or "tired" of only one kind of crop or also of various crops. In the former case an overcharge of only certain kinds of mycelium fungi and microbes may be the cause, in the latter an overcharge of fungus life in general. An illustration of the former case is furnished by vineyards which, after a series of years, begin to show a poor production in grapes even after liberal manuring. Formerly the practice was, in such a case, interrupt the culture of the vine, as young trees planted in the place of the old ones failed to grow; the practice was also to grow other crops as alfalfa, sometimes for fifteen years, or to leave the soil in fallow condition. In recent years, however, the application of bisulphid is practised in many vineyards with success. After removal of the old trees 4 to 6 holes, from 40 to 60 cm deep are made for each sq. meter; and bisulphid is applied varying in amount from 200 to 400 cc, according to the nature of the soil. The soil thus disinfected can serve after 5 to 6 weeks for planting to fresh young grapevines which show quick and healthy develo ment. Thus the vineyards are profitably maintained for the future.

It frequently occurs in vineyards that only here and there a vine shows the ill effect of "grape sick" soil. In this case the affected vine alone is removed and the soil treated with 100 to 120 cc bisulphid. This is done during the winter in order to prevent an unfavorable influence by the roots of the neighbouring vines After application the soil is puddled and pressed down in order to prevent a too rapid escape of the bisulphid by volatilization and to

secure a prolonged action in the soil.

Koch observed an increase of juice and sugar and a decrease of acid in grapes that had been grown after this treatment.

The following table shows the relation of sugar to juice after the application of varying amounts of bisulphid.

Bisulphid applied to one plant.	Sugar in 100 c.c. juice.	
o cc	21,6 grams	
25	25, 1	
50	23,0	
75	25, 5	

The application of bisulphid to live grapevines is also practised in France and Austria to destroy Phylloxera, but in this case not more than 24 grams bisulphid are applied to one sq. meter, distributed in 4 holes, 20 to 30 cm. deep, in order not to injure the live roots of the vines. The expense for one hectar amounts to from 25 to 35 dollars. In the Palatinate province of Germany bisulphid is extensively applied in vineyards where no parasites are present, as very favorable effects have been noticed by the farmers. Thus in the small district of Deidesheim alone 45,000 kilos of bisulphid more special in the respectation.

kilos of bisulphid were applied in the year 1905. (1)

Since the application of carbon bisulphid is rapidly increasing it may not be out of place to call attention to its inflamatory nature, danger existing even at low temperature. A match lighted at some distance or a burning cigar may lead to terrible consequences. The carbon bisulphid should be stored in well closed fire-proof vessels and in a cool place. The inhalation of the vapor causes anaesthesia. A suitable apparatus lately employed for its application consists of a well closed box, carried on the back, from which the liquid runs through a tube into a graduated injector, which is pushed into the ground and from which a definite quantity of bisulphid is discharged into each hole made by the injecting point. (2)

The introduction of soil disinfection in Porto Rico will doubtless lead to good results, especially since the continuous warm climate of tropical countries favors the rapid increase of microbes in soils. Disinfection might prove benificial every 3 or 4 years,

but this must be decided by further experiments.

Some determinations (3) by G. L. Fawcett of the number of mi-

(3) These determinations had to be made by means of agar plates, since galatine plates would have liquified too easily in this warm

climate.

⁽¹⁾ Cf. Hiltner, Praktische Blätter fur Pflanzenbau and Pflanzenschutz, 1909, pp. 45. Hiltner reports there also on the successful application of carbon bisulphid in killing grubs, various rodents, and other animals. Cresol and carbolineum mixed with humus or lime have been found still superior to bisulphid in certain cases.

⁽²⁾ Convenient forms are the McGowen injector which, however, does not seem to be on the market at present, and the Pal injecteur or Spritzpfahl manufactured at a cost of \$14.00 by the firm of Carl Platz in Ludwigshafen, Germany.

crobes in one gram of various Porto Rican soils may be mentioned in this connection, as follows:

No. of Soil.	No. of bacteria.
I	3,763,000
2	11,094,000
3	4,278,000
4	94,830,000
5	1,628,000
6	835,000

Of these soils only No. 4 had received barnyard manure annually, the other had received no organic manure recently, but

contained some debris of decaying vegetation.

An experiment on soil disinfection was carried out here by P. L. Gile (1) on plots planted to cane. Each plot of 16 sq. meter contained 4 holes and 300 cc bisulphid were applied to each hole. Four weeks after the application 4 cuttings of cane were planted over the spots that had received the bisulphid. The harvests one year later were as follows:

No bisulphid, no manure 268 lbs.
Bisulphid, no manure 345 "
Bisulphid, and manure 305 "
No bisulphid and manure 245 "

Hence, the bisulphid alone led to much better results than the full manure.

There occur in Porto Rico also cases of "sick" or "tired" soils. In various coffee plantations coffee trees commence to die a slow death without any fungus or animal parasites being discernible. The branches become at first yellow at the tip and the stratum of Chlorophyll bearing cells below the outer bark turns brownish. This phenomenon spreads gradually until the entire branch has dried up. The leaves of such trees often show a phenomenon noticed at the beginning of the Mosaic disease of tobacco, viz., a light green tissue between the veins and a dark green along the veins. These leaves gradually dry up and turn brown. The berries of such diseased coffee trees turn red before they are ripe. Such a condition was observed at the Aurelia plantation where on one side of a trail it was highly developed but not on the other side which was somewhat more inclined. The soil consisted of a red clay free of humus, almost devoid of calcium carbonate and with no animal life except a few earthworms. A microscopical examination of the soil surrounding the roots of sick trees did not reveal Nematodes. The specific odor of earth was very highly developed in that soil, an odor which, as Rullman has shown, is due to a peculiar microbe formerly named Cladothrix odorifera (later changed to Actinomyces

⁽¹⁾ This small experiment forms a part of one carried out on a larger scale, a full report of which will be prepared by Mr. Gile later on.

odorifera.) On shaking the soil with some distilled water and examining a drop after most of the mineral matter had settled, numerous bacteria could be recognized, but, since most soils contain a large number of bacteria this is not surprising. However, in close soils of limited aeration an accumulation of bacteria beyond a certain limit can no doubt act more injuriously by absorbing the air necessary for the respiration of the roots than in loose sandy soils. It is of considerable interest that, although this soil from its intensely red color apparently does not contain black humus, nevertheless contains organic matter, as is shown by the placken ing produced by heat. On heating there are also evolved strongly alkalin vapors which have the odor of decomposing proteins, and colored red litmus paper intensely blue. The bad condition of the trees could not have been due to any deficiency of nutrients in the soil since the proprietor of the plantation had already carried out a decisive manuring experiment for three years previous. There were six plots of 50 trees each, manured as follows:

I. Check plot.

II. 40 lbs. Tankage, 48 lbs Superphosphate; 1 lb. 8 oz. per tree III. 29 lbs. Dried Blood, 22 lbs. Potassium Chlorid; 1 lb. per tree IV. 53 lbs. Superphosphate, 32 lbs. Chlorid of Potassium; 1 lb.

8 oz per tree . 40 Tankage, 33 lbs. Superphosphate, 22 lbs. Chlorid of po-

tassium; 2 lbs. per tree.

VI. Check Plot.

The result was no essential change of the deplorable condition. The relatively best result was obtained on Plot II, but the yield was not equal to one-half that of the healthy trees on the unmanured soil nearby. The condition of the suffering trees led to the conclusion that the soil had attained an unfavorable condition, had become "tired" or "sick," especially for coffee. Larger trees, such as mangoes, did not apparently suffer on the same soil, but this may have been due to the fact that their roots penetrate to a greater depth. Since a soil disinfection seemed to be necessary, a trial was made with carbon bisulphid as well as with creolin, (1) the latter diluted to 5 times its volume. A report of the results will follow later on

⁽¹⁾ The 'creolin' is a liquid of a tar like odor, yielding a white emulsion with water and containing, according to an analysis furnished by the factory:

Cresols and pheno	1	2.67
Homologues of ber	nzene and other hydro	carbons 44.94
Organic bases		2.76
Resin		32.45
Sulphur		0.248
Water		5.34

This preparation, also called Cresoline or Sanatol, may be of similar composition as the "Carbolineum" which recently was applied very successfully to destroy Nematodes in the field, further as a disinfectant of soils, and for spraying purposes.

In order to see whether it would be necessary to remove the trees and to plant fresh ones after the disinfection or whether the suffering trees might recover by a direct application of carbon bisulphid, as has been practised in certain vineyards, a dose of 250 cc. carbon bisulphid was admi istered through a hole about one foot deep, made near the trunk. A former experiment with a healthy coffee tree had shown that no injury resulted when 50 cc of carbon bisulphid was applied to each of 5 holes, 6 to 9 inches deep, made in distances of 6 to 9 inches from the trunk.

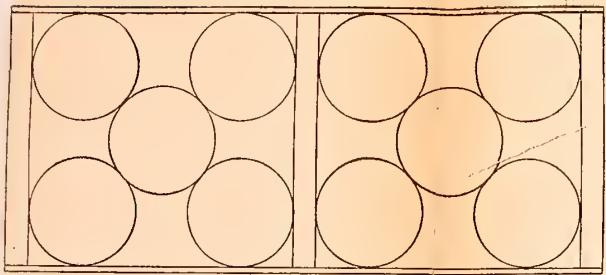
There exist various other soils in Porto Rico which are improperly called "worn out." They may have been long in use without application of manure, but since even rich manuring fails to produce satisfactory results, the su position seems justified that they contain an overcharge of bacteria. The application of the principle of soil disinfection may help to restore the former fertile

condition. Experiments will be made in this direction also.

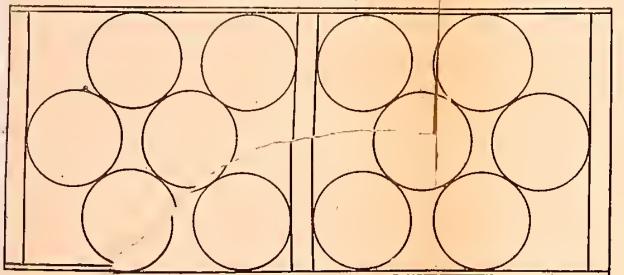
NOTE—In case the Pal injector is used, attention must be called to the undue rise of temperature to which the metallic container of the bisulphid, carried on the back, may be subjected by exposure to the direct rays of the sun. By this exposure the bisulphid may cause an explosion by the expansion of its vapor. The container should, therefore, be wrapped with a thick cloth which should be kept damp.

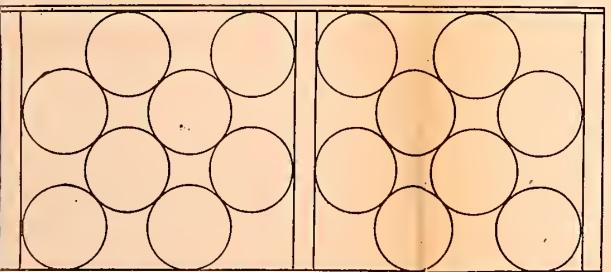


POMELOS AND ORANGES.



(Fla.) Size 28. 3 L. 1st. & 3rd.-5. 2nd. L-24. The Horticultural Society adopted this 5x4 and 4x5 of pack for all sizes up to 64.





(Cal.) Size 48. 3 L. 8 in each Layer. Also (Fla.) Size 64 Diam. about 41/4

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AGRICULTURAL EXPERIMENT STATION

MAYAGUEZ, P. R.

MARCH 1909.

L-layer (Fla.)-Florida only (Cal.-California only. (Fla & Cal)-both the same. First two layers with [brand side down, rest with

brand side up.

Dimensions of Fla. box

12 x 12 24 3/8 inches inside meas.

Dimensions of Cal. box

11 1/2 x 11 1/2 x 24 inches inside fruit space.

Lemon box same in both Fla. & Cal. 101/2 x 14 x 251/8 inches inside meas.

L- Camada (Fla.)-Florida solamente. (Cal.) solamente. (Fla. y Cal.)—el mismo de las dos,

Las primeras dos camadas con la marca hacia abajo, el resto con la marca bacia arriba.

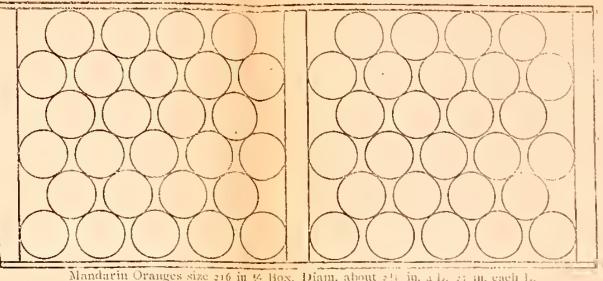
Tamaño de la caja de Florida:

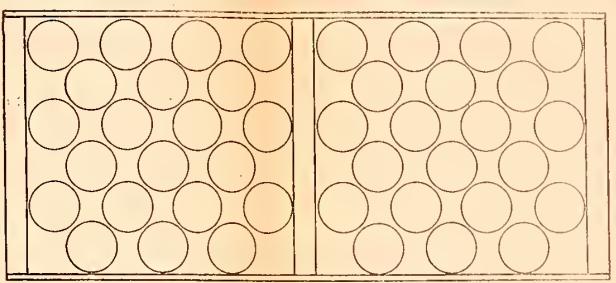
12 x 12 x 24 1/2 pulgadas medida interior.

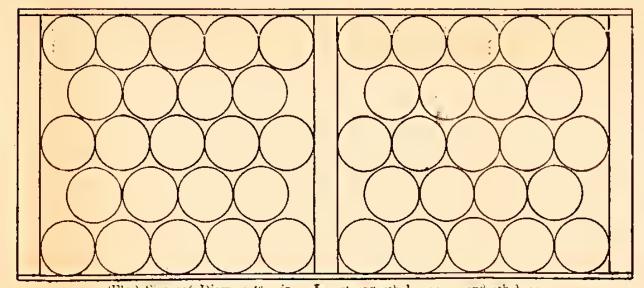
Tamaño de la caja de California:

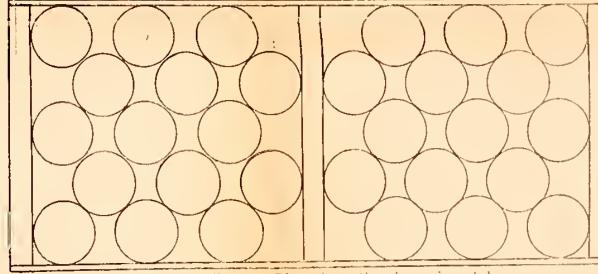
111/2 x 111/2 x 24 pulgadas volúmen interior.

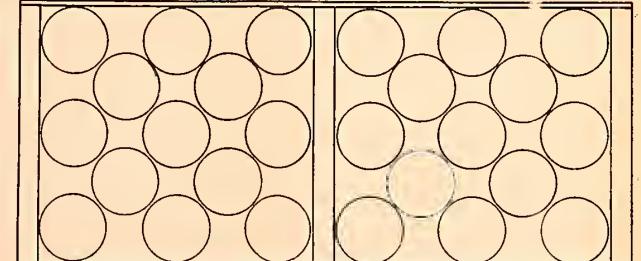
Caja para limones igual la de Fla. y Cal. 101/2 x 14 x 2558 pulgadas medida interior.











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